Minimally invasive aortic banding in mice: effects of altered cardiomyocyte insulin signaling during pressure overload


The ability to manipulate gene expression in mice has contributed greatly to the study of many diseases. In terms of the heart, the response of genetically altered animals to increased cardiac work (e.g., pressure overload) is of particular interest. Rockman and colleagues (33) pioneered a model of transverse aortic constriction in mice. The use of this model has provided significant insight into the cellular and molecular pathways responsible for the development of left ventricular (LV) hypertrophy (LVH). Although transverse aortic constriction in mice is now done routinely by a number of groups, the technical difficulty of the surgical procedure has limited the availability of this model.

Previously published methods for the creation of transverse aortic constriction in mice require microsurgical skills and the ability to provide mechanical ventilation when the thorax is entered. The requirement for tracheal intubation and low-volume, high-rate mechanical ventilation mandates additional time and expense associated with these procedures. Moreover, inflammatory reactions within the chest may complicate the analyses of cardiac function and pathology. Herein, we report a minimally invasive transverse aortic banding (MTAB) procedure in mice that obviates the need for providing mechanical ventilation because the pleural space is not entered. The procedure can be performed rapidly and with low mortality. Our approach produces consistent and sustained increases in LV pressure of 40–60 mmHg.

Signaling through the insulin and the insulin-like growth factor-I (IGF-I) pathways regulate developmental and postnatal somatic growth (41). Cardiac growth also appears to be regulated by downstream intermediates in the insulin/IGF-I signaling pathway. In particular, there is strong evidence that activation of phosphatidylinositol 3-kinase (PI3K) and/or the serine-threonine kinase Akt promotes cardiac growth (35–37). Conversely, we have previously shown that mice with cardiomyocyte-specific knockout of the insulin receptor (CIRKO) have reduced heart weight, reduced size of cardiac myocytes, and reduced phospho-Akt, as do mice with expression of dominant negative PI3K (9, 35, 37). CIRKO mice with forced expression of Akt have “rescue” of the small heart phenotype (37). Thus there is strong evidence that activation of the insulin-signaling cascade is important for physiological growth and development of the heart. However, it is not known whether insulin signaling regulates the development of hypertrophy in response to mechanical loading in adult cardiac myocytes. We applied the MTAB technique to test the hypothesis that lack of insulin receptors in the heart would attenuate the development of LVH in...
response to pressure overload. Our data show that hearts from CIRKO mice hypertrophy appropriately in response to pressure loading; however, they have greater LV dilatation, increased fibrosis, increased connective tissue growth factor (CTGF) expression, and reduced systolic function. These findings are compatible with the idea that divergent signals control cardiac growth and cardiac contractility (15).

**METHODS**

The institutional animal care and use committee approved all studies. Mice were cared for according to the “Guiding Principles for Research Involving Animals and Human Beings.”

An initial set of studies was performed to validate the MTAB technique. Male C57/BL6 mice weighing 20–30 g were used in these experiments. Mice were anesthetized with a single intraperitoneal injection of chloral hydrate (400 mg/kg). A topical depilatory agent was applied to the neck and chest, and the area was cleaned with betadine and alcohol. Mice were placed supine, and temperature was maintained at 37°C with a heating pad. A horizontal skin incision ~0.5–1.0 cm in length was made at the level of the suprasternal notch. The thyroid was retracted, and a 2- to 3-mm longitudinal cut was made in the proximal portion of the sternum. This allowed visualization of the aortic arch under low-power magnification. A wire with a snare on the end was passed under the aorta between the origin of the right innominate and left common carotid arteries. A 6-0 silk suture was snared with the wire and pulled back around the aorta. A bent 27-gauge needle was then placed next to the aortic arch, and the suture was snugly tied around the needle and the aorta. After ligation, the needle was quickly removed. The skin was closed, and mice were allowed to recover on a warming pad until they were fully awake. The sham procedure was identical except that the aorta was not ligated.

Echocardiography was performed 7 and 14 days after MTAB or sham surgery. During the validation study, lightly anesthetized mice (chloral hydrate) were imaged in the left lateral decubitus position with a linear 13-MHz probe (General Electric, Vivid V echocardiograph). Digital images were obtained at a frame rate of 180 images/s. Two-dimensional images were recorded in parasternal long- and short-axis projections with guided M-mode recordings at the midventricular level in both views. LV wall thickness (intraventricular septum [IVS] and posterior wall [PW] thickness) and internal dimensions at diastole and systole (LVId and LVIDs, respectively) were measured. LV fractional shortening ([LVIDd − LVIDs]/LVIDd), relative wall thickness ([IVS thickness + PW thickness]/LVIDd), and LV mass (1.05 [IVS thickness + LVIDd + PW thickness]3 − LVIDd3) were calculated from the M-mode measurements. After the final echocardiogram, LV pressure was measured with a 1.4-Fr micromanometer-tipped catheter (Millar Instruments) inserted through the right carotid artery.

After hemodynamic recordings were completed, an additional dose of chloral hydrate was given. With the mice under deep anesthesia, the hearts were rapidly excised, trimmed of fat, gently blotted dry, and weighed. Hearts were first perfused via the aorta at physiological pressures with 4% paraformaldehyde. Hearts were subsequently immersed in paraformaldehyde for 48–72 h, embedded in paraffin, and then processed for histology.

In a subgroup of animals (n = 3), LV pressure was measured immediately after the MTAB procedure and after release of the ligature around the transverse aorta (acute banding). In another subgroup of animals (n = 3), the heart and aorta were perfused through the LV apex with a liquid solution of latex. After the latex hardened, the aorta was removed to show the location of the band and the degree of constriction.

After the MTAB technique had been validated, we used this approach to study the role of insulin signaling in the regulation of cardiac hypertrophy and function during pressure overload. CIRKO mice were compared with wild-type (WT) littermate controls. The CIRKO mice were created on a mixed FVB/SVJ/C57 background (9). Adult CIRKO mice are known to have smaller hearts (~20% lower heart weight) than WT mice (9, 37). CIRKO mice have normal systemic glucose homeostasis, normal body size, and normal longevity. CIRKO (n = 4) and WT mice (n = 5) underwent MTAB as described above. Sham-operated mice (CIRKO, n = 4; WT, n = 3) served as controls. In this portion of the study, echocardiograms were performed in conscious mice 2 and 4 wk after MTAB or sham surgery. Hemodynamic measurements were subsequently performed under anesthesia as described above.

In a subgroup of animals, hearts were placed in RNAlater (Ambion; Austin, TX) and then frozen at −80°C. Tissues were homogenized, and total RNA was extracted by using TRIzol reagent (Invitrogen; Carlsbad, CA) according to the manufacturer’s recommendations. Chloroform was added to the homogenate, and the RNA-containing aqueous phase was further purified using the Qiagen RNAeasy total RNA isolation kit (Valencia, CA) according to the manufacturer’s instructions. Three micrograms of each total RNA was synthesized to cDNA using Superscript TMII RNase H-Reverse Transcriptase (Invitrogen) using the manufacturer’s protocols and oligo dT primers. Quantitative real-time PCR was performed with 8 ng of cDNA as the template. Final concentrations of other PCR reagents were as follows: 0.5 mM each primer, 200 mM each dNTP, 50 mM Tris (pH 8.3), 500 mg/ml nonacetylated BSA (Sigma; St. Louis, MO), 3.0 mM MgCl2, 0.04 U/ml Platinum Taq DNA polymerase (Invitrogen), and 1:30,000 dilution (in sterile water) of SYBR Green I fluorescent dye (Molecular Probes; Eugene, OR). Primers were designed from mouse cDNA sequences available in GenBank and were designed to span at least two exons of the genomic locus. Internal standard curves were made using a pool of all cDNA template samples for a given transcript. Quantification of cDNAs of interest was performed with LightCycler software (Roche Diagnostics; Indianapolis, IN). For each sample’s fluorescence versus cycle line, the second derivative maximum (the “crossing point” or threshold cycle at which the fluorescence clearly increases above background fluorescence) was determined. Amplification employed 40 four-step cycles with a rate of temperature change between steps of 20°C/s. Transcript levels for the constitutive housekeeping gene product cyclophilin were also quantitatively measured in each sample, and PCR data are reported as the number of transcripts per number of cyclophilin molecules. Data were additionally normalized to the mean WT value.

Primer sequences used were as follows: atrial natriuretic factor (ANF), sense 5'-GAGAGACGCAGCTTCTTGGAC-3' and antisense 5'-CTGGACACACCACAAAGGGCTTGG-3'; β-myosin heavy chain (MHC), sense 5'-GACGAGCCAGAGCAGCAGTGC-3' and antisense 5'-GGGCTTCAAGGAGCTTCTGCAG-3'; CTGF, sense 5'-AAGGACAGGTCGACAAATACCA-3' and antisense 5'-GGCCAAATGGTCTTTCCAGT-3'; and cyclophilin, sense 5'-AGCACTGGAGAAGGAGTGTGG-3' and antisense 5'-TCTTCTTGGCTGCTTGGCCATT-3'.

**Statistics.** Data are shown as means ± SE. In the validation study, comparisons between sham and MTAB mice were analyzed by 1-way ANOVA followed by the Tukey-Kramer test.
performed using a two-tailed, unpaired Student’s t-test. In the second portion of the study, factorial ANOVA was used to assess differences between groups. When appropriate, ANOVA was followed by a Fisher’s protected least-significant difference test for specific intergroup comparisons (Statview 5.01, SAS Institute; Cary, NC). The data for the mRNA expression were not normally distributed, so a nonparametric test (Mann-Whitney U-test) was used for analysis of these data. Only the most relevant comparisons between groups are shown. These include banded and sham-operated animals of the same genotype (i.e., WT band vs. WT sham and CIRKO band vs. CIRKO sham) or the same treatment in two genotypes (i.e., CIRKO sham vs. WT sham or CIRKO band vs. WT band). A probability of ≤0.05 was considered to be significant.

RESULTS

To validate the MTAB procedure, 11 mice underwent sham surgery and 16 mice had MTAB. One mouse died acutely after being banded. No sham-operated animals died. Three banded mice underwent LV pressure measurement immediately before and after being banded. LV systolic pressure acutely increased from 88 ± 4 to 146 ± 9 mmHg (P < 0.05 vs. baseline). Of the 12 remaining banded mice, one was killed at 7 days because of markedly reduced LV function seen on echocardiography. Eleven banded mice from the initial cohort were euthanized at 14 days after being banded. An example of a mouse aorta after chronic aortic banding is shown in Fig. 1. The location of the ligation between the innominate and left carotid origins is evident. Measurement of the narrowest diameter of the aortic cast (0.4 mm) was nearly identical to the diameter of the 27-gauge needle. Measurements of the cross-sectional areas of the latex cast suggested a ~90% reduction in the cross-sectional luminal area of the aorta. Similar results were seen in three banded mice.

Sustained increases in LV systolic pressure were seen after 2 wk of banding (Table 1). Echocardiography at 14 days postsurgery revealed increased LV wall thickness, increased LV mass, no change in LV cavity size, and no change in LV systolic function in the banded mice (Table 1). Two weeks after surgery, heart weight and heart weight/tibia length were increased in MTAB mice compared with sham-operated mice (Table 1).

After the validation study, the MTAB procedure was used to investigate the role of insulin signaling in the regulation of the cardiac response to pressure overload. Survival in banded CIRKO and WT mice was 100% (n = 4 and 5, respectively) and also 100% in shams (n = 4 and 3, respectively). Four weeks after MTAB, LV systolic pressure increased by the same amount (~60 mmHg) in WT and CIRKO mice (Table 2 and Fig. 2). Banded animals of both genotypes had comparable LV end-diastolic pressures that were both increased relative to their respective sham-operated controls (Table 2 and Fig. 2). Interestingly, the negative first derivative of LV pressure increased in both WT and CIRKO mice after they were banded, whereas the positive first derivative of LV pressure was unchanged. Similar changes were seen in the validation study (Table 1).

LV mass increased to a comparable extent in CIRKO and WT mice after they were banded (27% and 22% vs. respective controls; Table 3). However, the pattern of LV remodeling was different in CIRKO and WT mice. Serial echocardiograms revealed that banded CIRKO mice developed a greater degree of LV cavity dilatation than did banded WT mice (Table 3 and Fig. 3). Both

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Table 1. Echocardiographic and hemodynamic measurements and heart weights in sham-operated mice and mice with MTAB

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MTAB</th>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>431 ± 19</td>
<td>519 ± 22</td>
</tr>
<tr>
<td>IVS, thickness, mm</td>
<td>0.9 ± 0.02</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>PW thickness, mm</td>
<td>0.8 ± 0.05</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>LVIDd, cm</td>
<td>3.4 ± 0.1</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>LVIDs, cm</td>
<td>1.9 ± 0.2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>41 ± 1</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>91 ± 4</td>
<td>133 ± 11*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8.7 ± 1.6</td>
<td>11.8 ± 3.0</td>
</tr>
<tr>
<td>+dP/dt</td>
<td>6,204 ± 617</td>
<td>6,344 ± 760</td>
</tr>
<tr>
<td>−dP/dt</td>
<td>5,958 ± 556</td>
<td>7,487 ± 476*</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>142 ± 6.1</td>
<td>222 ± 20.5*</td>
</tr>
<tr>
<td>Heart weight/tibia length, g/cm</td>
<td>0.081 ± 0.003</td>
<td>0.134 ± 0.019*</td>
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</table>

Values are means ± SE; n, no. of mice. Measurements were made under anesthesia 14 days after minimally invasive transverse aortic banding (MTAB) or sham surgery. Left ventricular (LV) pressure measurements were only performed in 8 of 11 MTAB mice. HR, heart rate; IVS, interventricular septum; PW, posterior wall; LVIDd, LV internal diastolic dimension; LVIDs, LV internal systolic dimension; FS, fractional shortening; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; dP/dt, first derivative of LV pressure. *P < 0.05 vs. sham; †P = 0.06 vs. sham (2-tailed unpaired t-test) and P = 0.048 (Mann-Whitney U-test).

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Fig. 1. Latex casts of aortas after minimally invasive transverse aortic banding (MTAB; left) and sham surgery (right). The silk ligature has been removed, but the site of narrowing is evident (arrow demonstrates the location of constriction).
WT and CIRKO banded mice had increased LV wall thickness relative to their respective shams. However, banded WT mice had greater wall thickness than banded CIRKO. Banded CIRKO mice had no change in relative wall thickness at 4 wk compared with CIRKO shams. This was in contrast to the increase in relative wall thickness seen in WT mice 4 wk after they were banded. In conjunction with the divergent patterns of LV remodeling, LV systolic function (as assessed by fractional shortening) decreased significantly more in the banded CIRKO mice compared with the banded WT (Table 3 and Fig. 4).

The hearts from banded mice had markedly increased fibrosis (Fig. 5). Interestingly, the pattern of fibrosis was mainly perivascular in WT hearts compared with the more interstitial pattern in CIRKO hearts (Fig. 5). The interstitial fibrosis in banded CIRKO hearts was most evident in the subendocardium (not shown). In conjunction with the interstitial fibrosis seen on histology, CTGF expression increased significantly more in banded CIRKO hearts than in banded WT hearts (Fig. 6). β-MHC and ANF mRNA increased comparably after CIRKO and WT hearts were banded (Fig. 6).

DISCUSSION

Mechanical constriction of the aorta has been used for many years to produce pressure-overload hypertrophy in a number of different species. In general, the extent of hypertrophy and the likelihood of progressing to heart failure increases as the band is placed closer to the heart. Therefore, it is desirable to place the band on the thoracic aorta as opposed to the abdominal aorta. Rockman et al. (33) pioneered the approach of placing the band between the innominate and left carotid artery. This technique has the advantage of placing the constriction close to the heart but still allowing hemodynamic measurements to be made by retrograde passage of a catheter through the right carotid artery. The standard surgical approach to the transverse aorta has been to enter the chest through the second left intercostal space. This approach causes a breach of the pleural space and necessitates mechanical ventilation. The use of mechanical ventilation in mice requires additional time, expertise, and equipment.

We developed a minimally invasive technique for constricting the transverse aorta in mice that does not require mechanical ventilation. We found that we

Table 2. LV hemodynamics and heart weights 4 wk after MTAB or sham surgery in WT or CIRKO mice

<table>
<thead>
<tr>
<th></th>
<th>WT Sham</th>
<th>WT Band</th>
<th>CIRKO Sham</th>
<th>CIRKO Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>440 ± 43</td>
<td>486 ± 26</td>
<td>468 ± 24</td>
<td>488 ± 37</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>84 ± 4</td>
<td>145 ± 7*</td>
<td>93 ± 8</td>
<td>156 ± 5*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>6 ± 3</td>
<td>25 ± 4*</td>
<td>12 ± 4</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>5,985 ± 554</td>
<td>6,979 ± 328</td>
<td>6,793 ± 450</td>
<td>6,866 ± 453</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>5,880 ± 396</td>
<td>7,896 ± 297*</td>
<td>5,694 ± 624</td>
<td>7,337 ± 599*</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>143.7 ± 9.6</td>
<td>175.6 ± 13.1*</td>
<td>120 ± 3.0</td>
<td>186.1 ± 7.7*</td>
</tr>
<tr>
<td>Heart weight/tibia length, g/cm</td>
<td>0.085 ± 0.006</td>
<td>0.102 ± 0.007*</td>
<td>0.068 ± 0.001†</td>
<td>0.107 ± 0.004*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. Measurements were performed in anesthetized mice. WT, wild type; CIRKO, cardiomyocyte insulin receptor knockout. *P < 0.05 vs. sham of the same genotype; †P < 0.05 vs. WT sham. There were no significant differences in CIRKO band vs. WT band.
could consistently band the aorta in <10 min with rapid recovery and low morbidity and mortality. Not only is the approach substantially faster and less expensive, but potential confounding experimental factors such as inflammation within the chest and healing of the chest wall incision are reduced. We found changes in LV size and function and expression of hypertrophic markers (i.e., increased expression of ANF and β-MHC) that were very comparable to those achieved with the conventional surgical technique. The use of a technique with low mortality is important when studying the effects of pressure overload in rare or precious lines of genetically altered mice.

With the use of the MTAB technique in mice with absent cardiomyocyte insulin receptors, we found that impaired insulin signaling in cardiac myocytes is associated with a maladaptive pattern of LV remodeling during pressure overload. This may be expected to eventually accelerate the transition from compensated cardiac hypertrophy to heart failure. There is accumulating evidence that insulin action is impaired in the hearts of humans and animals with diabetes and insulin resistance (20, 25, 27). Moreover, the likelihood of developing heart failure is greater in subjects with diabetes and insulin resistance when there is concomitant hypertension (21, 22). Thus impaired insulin action in the myocardium may contribute to the synergistic adverse effects of diabetes and hypertension.

Cardiomyocyte insulin receptors are deleted shortly after birth in CIRKO mice. The reduced cardiac mass in these animals implies that insulin signaling participates in the physiological regulation of cardiomyocyte size (9). We (37) recently showed that one mechanism linking insulin signal transduction to the regulation of cardiomyocyte size in CIRKO mice was decreased activation of Akt. Thus we asked whether lack of insulin

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**Table 3. Echocardiographic measurements in conscious mice 4 wk after MTAB or sham surgery**

<table>
<thead>
<tr>
<th></th>
<th>WT Sham</th>
<th>WT Band</th>
<th>CIRKO Sham</th>
<th>CIRKO Band</th>
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<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>649 ± 55</td>
<td>688 ± 10</td>
<td>647 ± 34</td>
<td>617 ± 37</td>
</tr>
<tr>
<td>IVS thickness, mm</td>
<td>0.86 ± 0.09</td>
<td>1.28 ± 0.05*</td>
<td>0.82 ± 0.04</td>
<td>1.09 ± 0.06†</td>
</tr>
<tr>
<td>PW thickness, mm</td>
<td>0.9 ± 0.10</td>
<td>1.12 ± 0.10</td>
<td>0.85 ± 0.05</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.6 ± 0.07</td>
<td>3.6 ± 0.17</td>
<td>3.5 ± 0.15</td>
<td>4.2 ± 0.06†</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>1.7 ± 0.04</td>
<td>2.0 ± 0.20</td>
<td>1.7 ± 0.27</td>
<td>2.5 ± 0.13†</td>
</tr>
<tr>
<td>FS, %</td>
<td>53.6 ± 1.1</td>
<td>44.2 ± 3.3</td>
<td>52.5 ± 5.8</td>
<td>30.1 ± 2.3†</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>118 ± 20</td>
<td>181 ± 21*</td>
<td>105 ± 10</td>
<td>189 ± 14*</td>
</tr>
<tr>
<td>RWT</td>
<td>0.48 ± 0.05</td>
<td>0.67 ± 0.04*</td>
<td>0.47 ± 0.02</td>
<td>0.50 ± 0.03†</td>
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</tbody>
</table>

Values are means ± SE; n, no. of mice. RWT, relative wall thickness ([IVS thickness + PW thickness]/LVIDd). *P < 0.05 vs. sham of the same genotype; †P < 0.05, CIRKO band vs. WT band. There were no statistical differences between CIRKO sham and WT sham.
signaling would limit the hypertrophic response to extrinsic stimuli such as pressure overload. We found that heart weight and expression of the hypertrophic markers β-MHC and ANF increased comparably in mice with and without cardiomyocyte insulin receptors (Table 2 and Fig. 6). Therefore, insulin signaling does not appear to be mandatory for hypertrophy in adult hearts. However, the pattern of LV remodeling and the functional consequences of pressure overload were influenced strongly by the absence of cardiomyocyte insulin receptors. Despite the fact that CIRKO mice and WT mice achieved similar heart weights after they were banded, after 4 wk the CIRKO hearts were more dilated and systolic function was reduced compared with WT hearts. Relative wall thickness was also reduced in banded CIRKO hearts, implying higher LV wall stress.

The phenotype of the banded CIRKO heart bears some similarity to changes previously reported in the hearts of humans and animals with insulin resistance, obesity, or Type 2 diabetes. For example, cardiac hypertrophy and LV dilatation are well described in humans with obesity and in animal models of insulin resistance and obesity (4, 5, 14, 16, 18). Large epidemiological studies such as the Strong Heart Study showed that Type 2 diabetes was associated with LVH.
and systolic dysfunction that was independent of changes in blood pressure (18). Insulin resistance and obesity are associated with increased blood volume and increased activation of the sympathetic nervous system and potentially the renin-angiotensin system (3, 6, 32). Thus the possibility exists that the presence of neurohumoral and hemodynamic changes coupled with alterations in myocardial insulin signaling could contribute to or exacerbate the abnormal LV remodeling that characterizes these states.

The exact signaling mechanisms that contribute to the more rapid decompensation of the CIRKO heart in response to pressure-overload hypertrophy remain to be elucidated. LVH in response to pressure overload involves the activation of multiple signal transduction pathways such as MAPKs (ERK1/2, p38, and JNK), protein kinases (e.g., PKC), and the serine-threonine phosphatase calcineurin (30). Activation of the IGF-I-PI3K-Akt signal transduction pathway can also lead to cardiac hypertrophy, but it is less clear whether this pathway contributes to pathological hypertrophy. Insulin signal transduction intersects with some of the signaling molecules that have been implicated in the pathogenesis of pressure-overload hypertrophy. For example, ERK1/2 and p38 are phosphorylated after activation of insulin receptors (38, 41). More importantly, in other cell types, impaired signaling via PI3K-Akt can augment signal transduction via ERK1/2 (24, 31). Thus it is possible that increased signaling via the MAPK pathway could contribute to a more pathological form of LVH in CIRKO mice relative to controls.

It is generally believed that the transition from compensated LVH to overt cardiac failure is associated with myocardial cell loss due to necrosis and/or apoptosis (1, 19). The signaling mechanisms responsible for this transition are only partially understood. Indeed, it has been shown that increased activity of ERK1/2, JNK, and calcineurin could actually be antiapoptotic and may act to promote cardiomyocyte survival (10, 17, 34, 42). IGF-I signaling has been shown to be antiapoptotic in the heart (28, 29). Whether or not insulin signaling is antiapoptotic in vivo is less clear. Administration of insulin in pharmacological doses (in vivo and in vitro) in the context of ischemia-reperfusion has been shown to reduce cardiomyocyte apoptosis (23, 26). The possibility therefore remains that the findings in the CIRKO heart could indicate increased susceptibility of insulin-resistant hearts to apoptotic injury. Because the defect in insulin signaling in this model is at the level of the insulin receptor, and in light of previously published evidence that IGF-I responses in CIRKO cardiomyocytes are preserved (9), it is possible that IGF-I administration could potentially rescue or retard the cardiac dysfunction observed in banded CIRKO mice.

A metabolic basis for the impaired response of CIRKO hearts to pressure-overload hypertrophy also needs to be considered. Compensated LVH is associated with a shift in metabolism away from fatty acid utilization and toward glucose utilization (2). At the transcriptional level, this is associated with decreased expression/activity of peroxisome proliferator-activated receptor-α and the expression of genes involved in fatty acid oxidation (7, 8). There is also increased activation of AMP kinase and increased expression and plasma membrane content of glucose transporters, particularly GLUT1 (39). Although there is a shift away from fatty acid utilization, fatty acids still contribute ~55% of the energy requirements of the hypertrophied heart (2). CIRKO mice exhibit significant defects in both fatty acid and glucose oxidation and also have reduced expression of GLUT1 (9). Thus it is likely that an inability to utilize several key substrates limits energy production during pressure-overload hypertrophy in CIRKO mice. This could contribute to the development of cardiac dysfunction in this model.

While CIRKO hearts remain relatively well compensated under nonstressed conditions, the abrupt increase in workload after aortic banding may push the heart into an energetically unfavorable balance due to a limitation in the quantity or rate of ATP production. In this scenario, ATP depletion leads to cellular con-
tractile failure with subsequent LV dilatation and global systolic dysfunction. In addition, a subset of cells might undergo necrotic cell death if ATP depletion becomes severe. The finding of increased interstitial and patchy fibrosis in banded CIRKO hearts, particularly in the subendocardium, supports this mechanism (i.e., cellular injury due to inadequate ATP). Because the subendocardium is a watershed region in terms of vascular perfusion, it is often the most susceptible region to ischemic damage if banded CIRKO hearts are relatively depleted of ATP, repetitive or transient episodes of subendocardial hypoperfusion might result in more severe ischemic damage than in hearts with adequate stores of high-energy phosphates. In addition to limited energetic reserves, the predominance of subendocardial hypoperfusion might result in more severe ischemic damage than in hearts with adequate stores of high-energy phosphates. In addition to limited energetic reserves, the predominance of subendocardial hypoperfusion might result in more severe ischemic damage than in hearts with adequate stores of high-energy phosphates. In addition to limited energetic reserves, the predominance of subendocardial hypoperfusion might result in more severe ischemic damage than in hearts with adequate stores of high-energy phosphates. In addition to limited energetic reserves, the predominance of subendocardial hypoperfusion might result in more severe ischemic damage than in hearts with adequate stores of high-energy phosphates.

In summary, we developed a minimally invasive approach for banding the transverse aorta in mice. This technique should substantially increase the availability of a reproducible LV pressure-overload model. Moreover, our observations support the testable hypothesis that reduced activation of intermediate molecules in the insulin signaling pathway directly exacerbates cardiac dysfunction in the context of increased pressure or other forms of cardiac overload. If correct, this hypothesis might help to explain the poor outcomes in diabetic patients with a variety of cardiovascular diseases (11, 12).

DISCUSSIONS

This study was supported by grants from the Department of Veterans Affairs, National Institutes of Health Grants HL-52338-06, U01-70525, T32-HL-57576, and DK-58073, the American Diabetes Association, and the Ben and Iris Margolis Foundation.

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